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(54) **ATP binding cassette transporter 1 (ABC1) gene polymorphisms and uses thereof for the diagnosis and treatment of lipid disorders, cardiovascular diseases and inflammatory diseases**

(57) The present invention provides four common polymorphisms in the gene encoding the ATP-binding cassette transporter-1 (*ABC1*), which are associated with decreased ApoA-I mediated efflux of cholesterol, the first step of reverse cholesterol transport. The provided data can be taken as evidence that common polymorphisms in *ABC1* directly affect cellular lipid homeostasis, which is a key factor in the atherogenetic proc-

ess. The frequencies of three of the common *ABC1* variants are significantly increased in a population of men having low HDL-C levels and established CHD relative to CHD-free control subjects. The use of the provided *ABC1* polymorphisms for the diagnosis and treatment of lipid disorders, cardiovascular diseases, and inflammatory diseases like psoriasis and lupus erythematosus is claimed.

Figure 2: human *ABC1* gene – protein translation

```

1      AAACCCCTTAATTGCGAGCGGAGTGTAGTGSGGCCGGGACC CGCAGAGGC   50
    +-----+-----+-----+-----+-----+-----+-----+
51     GAGCCGACCCCTTCTCTCCCGGGCTGCGCAGGGCAGGGCGAGTCCTCG   100
    +-----+-----+-----+-----+-----+-----+-----+
101    CGTACCAACAGAGCGGGTTCTCACGGCGCTTTGTCTCTTGTTTTTCTCCC   150
    +-----+-----+-----+-----+-----+-----+-----+
151    GGTTCTGTTTTTCTCCCGCTTCTCCGGAAGGCTTGTCAAGGGGTAGGAGAAA   200
    +-----+-----+-----+-----+-----+-----+-----+
201    GAGACGCAAACACAAAGTGGAAAAACAGTTAATGACCAGCCACGSCGTCC   250
    +-----+-----+-----+-----+-----+-----+-----+
251    CTGCTGTGAGCTCTTGGCGGTGCTCTCCAGGGCTCCCGAGCCACAGCTG   300
    +-----+-----+-----+-----+-----+-----+-----+
301    CGCGTCTGCGCTGAGGGGAACATGKCTTGTGTGGGCTCAGCTGAGGTTGCTG   350
    +-----+-----+-----+-----+-----+-----+-----+
                                     M A C K P Q L R L L
351    CTGTGGAAGAACCTCACTTTCAGAAGAAGACAaaacatgtcagctgttact   400
    +-----+-----+-----+-----+-----+-----+-----+
L W K N L T F R R R Q T C Q L L L
401    ggaaggggcgctggccctctatttatcttcctgatcctgatctctgttgctg   450
    +-----+-----+-----+-----+-----+-----+-----+
E V A W P L F I F L I L I S V R L
451    tgagctaccacccctatgaacaacatgaatgccattttccaataaagcc   500
    +-----+-----+-----+-----+-----+-----+-----+
S Y P P Y E Q H E C H F P N K A
501    atgcacctgcaggaaacacttccttgggttcaggggattatatctgtaatgc   550
    +-----+-----+-----+-----+-----+-----+-----+
M P S A G T L P W V Q G I I C N A
551    caacaacccccgtcccggttacccgactctggggaggctcccgaagtgtg   600
    +-----+-----+-----+-----+-----+-----+-----+
N N P C F R Y P T P G E A P G V V

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651  aggtctctttatccagccagaagacccagatgaaggacatgcgcaa
    R L L L Y S Q K D T S M K E H R K -
701  agttctgagacattacagcagatccagaatccagcccaacttgagc
    V L R T L Q Q I F K S S S N L K L -
731  ttcaagatttctgggtggacaacgaacctctctgggttccgtatcac
    Q D F L V D N B T P S G F L Y H -
801  aacctctctcccaagctctactgtggacaagatgctgagggctgatgc
    N L S L P K S T V D K H L R A D V -

851  cattctcccaaggtattttgcaaggctaccagttacatttgacaagtc
    I L H K V F L Q G Y Q L H L T S L -
901  tggcaatcgatcaaaacagagagatgattccactgggtgaccaagaa
    C N G S K S E H X I Q L G D Q E -
951  gttctgagcttttgggtctaccagggaagaactggctgacggcagcg
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1001  agtacttctgtccacatggacactggaagccactctgagaacactaa
    V L R S N M D I L K P L R T L N -
1051  archetacacttcccttcccgagcagcagctggccgaagccacaanaa
    S T S P P F S K E L A B A T E T -
1101  ttgctgcatagtcttgggactctggccaggagctgttccactgagaag
    I L H S L G T L A Q E L F S M R S -
1151  ctggagtgacatggcagcaggaggtgatgttttgcacaaatggaacagc
    W S D M P Q E V M P L T H V N S S -
1201  ccagctctccacccaatctacaggcgtgtgtctgtatctgtctgggg
    S S E T Q I Y Q A V S R I V C G -
1251  catcccgagcagggggagctgaagatccagctctcactggatgagga
    H P G G C C L K T X S L N W Y E D -
1301  caacaactacaagccctcttggggagcagggcactggagagagatgcg
    N N Y K A L P Q G N G T E E D A E -
1351  aaacctctctgacaactctacaactcttaactgcaatgatttgatgaag
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1401  aatttggagtcagtcctcttcccgattatctggaagctctgaagcc
    N L E S S P L S R I I W K A L K P -
1451  gctgctgttgggaagatcctgtatacactgacactccagccacaagcc
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1501  aggtcatggctgaggtgaacaagaccttccaggaactggctgtgtccat
    V M A E V N K T F Q E L A V F H -
1551  gatctggaaggcatgtgggaggaactcagcccaagatctggaccttcac
    D L E G H W E E L S P K I N T F N -
1601  ggagacagccagaagaatggacctgttcgggatgctgtgtgacagcagg
    E N S Q E M D L V R M L L D S R E -
1651  acaatgaccacttttgggaacagcagttggatggcttagattggacagcc
    N D E F W E Q Q L E G L D W T A -
1701  ccagacatctgtggcttttggccaaagccagaggatgtcaggtccag
    Q D I V A F L A K H P E D V Q S S -
1751  taatgggttctgtacaccggagagaagctttcaacagactaacccagc
    N G S V Y T W R E A F N E T N Q A -
1801  caatccggaccatctctgcttcatggagtggtgcaactgaacaagcta
    I R T I S R F M E C V N L N K L -
1851  gaacccatagcaacagaagctctggctcatcaacaaagtcacatggagctgc
    R F I A T E V W L I N X S M E L L -
1901  ggatgagaggaggttctgggtctggtattgttctcactggaattactcag
    D E R K F W A G I V F T G I T P G -
1951  gcaagattgagctgccccatctgtcaagtacaagatccgaatggacatt
    S I E L P H H V K Y K I R M L I -
2001  gacaatgtggagaggacaaataaaatcaggatgggtactgggacccctgg
    D N V E R T N K I K D G Y W D P G -
2051  tctcagagctgaccccttggagcagtcgggtacgtctgggggggtctcg
    P R A D P F E D M R Y V W S G F A -
2101  ctacttgcagagatgtgtggagcagcgaatcatcagcgtgctgacgggc
    Y L Q C V V E Q A I I R V L T G -
2151  acogagaagaaactggtgtctatgtgcaacagatgcctatccctgtta
    T E K X T G V Y M Q Q M P Y P C Y -
2201  cgtgatgacatcttctgagggtgatgagccgggtcaatgcccctcttca
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2250

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4001 -----+-----+ 4050
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      tggagaataattccctcaagtggtcgaagagatggggtggatgctgag
4051 -----+-----+ 4100
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4101 -----+-----+ 4150
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4151 -----+-----+ 4200
      K Q E C L R P P T E D D A A E P K -
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4201 -----+-----+ 4250
      C S D I D P E S R E T D L S Q -
      atggatggcaagggtctaccagctgaagggtggaaacttacacagca
4251 -----+-----+ 4300
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4301 -----+-----+ 4350
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4351 -----+-----+ 4400
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4401 -----+-----+ 4450
      A L V F S L I V F P P F G K Y P S L -
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4451 -----+-----+ 4500
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4601 -----+-----+ 4650
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      K T Y V Q I I A K S L K N K I W V -
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 5701 K L B N I N B I L X S V P L I E -  
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 5851 S W U L V Q R H L Y A K A V E Q -  
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 5901 V V P P L I T V L I Q Y R F F T R -  
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 6151 L D V N G A C N S S T F K M L T -  
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 6201 G O I T V T R G D A F L H H N S I -  
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 6251 L S N I E E V H Q N M G Y C P Q F -  
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 6301 D A I T H L I T G K E H V E F F -  
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 6351 A L L R G V P B K E V G K V G R N -  
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 6401 -----+----- 6450

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 6551 X A R R F L N N C A L S V V K E G -  
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 6601 R S V V L T S H S N E E C B A L -  
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 6751 T A G S N P D A K R V Q C P F G -  
 ctgcatcttctggagctgttccaaagagaaccccgagcactgtctaca  
 6801 L A F P G S V P K R K K R N N L Q -  
 ataccagcttccatcttcaattatcttcttggcaggaattatcagcactcc  
 6851 Y Q L P S S L S S L A R I F S C L -  
 tctccagagcacaagcagcactccacatagaagctactctgttctcag  
 6901 S D S K K R L H T S D Y S V S Q -  
 acaacacttgaccagctatttgtgaactttgcccaaggaccaaagctatga  
 6951 T T L D Q V F V N H A E Q Q S D D -  
 tgaacacttcaagacctctcatcacacaaaacagacagtagtgagcag  
 7001 D H L K D L S L H K N Q T V V D V -  
 ttgcagcttccacatcttcttccagcagcagcagcagcagcagcagcagc  
 7051 A V L T S P L Q D S K V K E S Y -  
 ghtgaagaactcgtgttcaacggggtgctgaagcaagagggagctag  
 7101 V -  
 acttctctttgcaacatgtgaagctgttctggagcaagagcagaagcttg  
 7151 -----+----- 7200  
 atgtgggagagaagtcaactgggatactgtcactgatactattcaatgcaatg  
 7201 -----+----- 7250

caatttaattg

7251 -----+ 7260

**Description****Background of the invention**

[0001] Epidemiological studies have established that plasma high density lipoprotein cholesterol (HDL-C) concentrations are inversely associated with the incidence of coronary heart disease (CHD) (1-4). Moreover, HDL deficiency is the most common lipid abnormality observed in patients with premature CHD (5,6), with nearly half of all CHD patients having low concentrations of HDL-C. Genetic factors account for approximately 50% of the variation in HDL-C levels in the general population (7), with several common mutations identified in genes encoding for proteins involved in the regulation of plasma HDL-C concentrations and, ultimately, playing a role in the development of CHD (8).

[0002] Recently, we (9,10) and others (11-15) demonstrated that familial HDL deficiency (FDH) syndromes with either splenomegalie or atherosclerosis (e.g. Tangier disease (TD); familial hypoalphaglycoproteinaemia) are rare HDL-deficiencies caused by mutations in the gene encoding the ATP-binding cassette transporter-1 (ABC1). ABC1 is a member of the ATP binding cassette family of proteins that are involved in the transmembrane transport of a variety of different molecules, including bile acids, steroid hormones, ions, amino acids, sugars and vitamins (16-19). TD is characterized by severely diminished plasma HDL-C concentrations, reduced levels of low density lipoprotein cholesterol (LDL-C), the deposition of cholesteryl esters in the reticulo-endothelial system with splenomegaly and enlargement of tonsils and lymph nodes (20), as well as a predisposition to neuropathy and CHD (21,22). The HDL deficiency in these patients has been related to a rapid catabolism of HDL and a decrease in ApoA-I induced lipid efflux from various cells including mononuclear phagocytes (23-25). Familial hypoalphaglycoproteinaemia represents the other clinical phenotype that is characterized by a similar disturbance in ApoA-I metabolism but is associated with an enhanced risk of CHD rather than splenomegalie. ApoA-I mediated cellular cholesterol efflux is the first step in the reverse cholesterol transport and prevents excessive cholesterol accumulation in peripheral cells. In addition, this process is suggested to contribute to the formation of mature HDL particles, thereby influencing the pool size of HDL in the plasma.

[0003] Although mutations in *ABC1* cause TD, the precise molecular mechanism by which they do so remains unknown. Also, little is known about the association of ABC1 and HDL metabolism in individuals not affected with TD. Previous investigations on the function of ABC1 in mice have revealed its role in the engulfment of apoptotic cells by macrophages (26) and in macrophage interleukin-1 $\beta$  secretion (27). We have further shown that ABC1 is a cholesterol-responsive modulator of cellular lipid efflux (10) and, thus, plays a significant role in cholesterol homeostasis. Moreover, the ABC1-knockout mice (ABC1<sup>-/-</sup>) show massively reduced levels of serum lipids and lipoproteins. The expression of ABC1 in mucosa cells of the small intestine and the altered lipoprotein metabolism in ABC1<sup>-/-</sup> mice allows the conclusion that ABC1 plays also a major role in intestinal resorption and translocation of lipids into the lymph-system.

**Summary of the invention**

[0004] Four different polymorphisms in *ABC1* (G596A, T1136C, A2589G, and G3456C) were identified in different Tangier kindreds as well as 516 healthy control subjects. Each of these polymorphisms affect the primary structure of the gene product.

[0005] A frequent coincidence of the G596A and A2589G polymorphisms was identified indicating that those two mutations are in linkage disequilibrium.

[0006] The two most common polymorphisms (G596A and A2589G) are both associated with a decreased in vitro ApoA-I mediated efflux of cholesterol from mononuclear phagocytes emphasizing the role of ABC1 in lipid efflux, the first step in reverse cholesterol transport. These results also are evidence that common polymorphisms in *ABC1* directly affect cellular lipid homeostasis, which is a key factor in the atherogenic process. A reduction of ApoA-I mediated efflux of cholesterol from mononuclear phagocytes is also a typical feature of Tangier disease.

[0007] Plasma lipoproteins, and HDL-cholesterol in particular, are not affected, probably because the effect of the *ABC1* polymorphisms on the protein function is compensated by other factors controlling plasma HDL levels.

[0008] Three common variants in the gene encoding ABC1 (G596A, A2589G, and G3456C) occur with significantly increased frequency in a population of men having low HDL-C as their primary lipid abnormality and established CHD, providing evidence for a role of *ABC1* in the premature CHD associated with common HDL deficiency states.

[0009] In summary, the identified polymorphisms in the ABC1 gene can be used for diagnosis and therapy of lipid disorders, cardiovascular diseases, and inflammatory diseases.

**Abbreviations****[0010]**

5	ABC	ATP-binding cassette
	ANC	Anchor
	ApoA	Apolipoprotein A
	ApoB	Apolipoprotein B
10	ApoE	Apolipoprotein E
	ATP	Adenosine triphosphate
	BMI	Body mass index
	bp	Base pairs
	cAMP	Cyclic adenosin monophosphat
15	cDNA	Copy DNA
	CH	Cholesterol
	CHD	Coronary heart disease
	DET	Detection
20	DPM	Disintegrations per minute
	E-LDL	Enzymatically modified LDL
	f	Forward
	Fluo	Fluorescein
	FOS	Framingham Offspring Study
25	HDL	High density lipoprotein
	HDL-C	HDL-cholesterol
	kDa	Kilo Dalton
	LDL	Low density lipoprotein
30	LDL-C	LDL-cholesterol
	M-CSF	Macrophage colony stimulating factor
	ph	Phosphorylation
	r	Reverse
	SD	Standard deviation
35	SDS	Sodium dodecyl sulfate
	TD	Tangier Disease
	TG	Triglycerides
	VA-HIT	Veterans Affairs Cooperative HDL Cholesterol Intervention Trial
40	wt	Wild type

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### **Description of Tables:**

#### **Table 1:**

**[0012] Summary of the characteristics of the study population consisting of 516 healthy blood donors.** Data are expressed as mean  $\pm$  SD. n indicates the number of analyzed individuals.

#### **Table 2:**

**[0013] Frequency of ABC1 polymorphisms G596A, A2589G, G3456C, and T1136C in healthy control subjects.** Genotyping was performed by LightCycler (Roche Diagnostics) PCR analysis. Data provide the relative percentage of homozygous, heterozygous or wild type individuals at the different polymorphic sites. n indicates the number of analyzed individuals.

#### **Table 3:**

**[0014] Distribution of G596A and A2589G polymorphisms in healthy control subjects.** Data represent absolute numbers of subjects with the respective allele combination. Data in parenthesis provide the relative percentage within each group.

#### **Table 4:**

**[0015] Amplification primers and hybridization probes for the LightCycler polymorphism analysis.** Hybridization probes are designated as detection (DET) or anchor (ANC) probes. As indicated, they are labeled with LightCycler-Red 640 or 705 and fluorescein, respectively. Phosphorylation (ph) of the 3'-end prevents the elongation of the detection probe. All detection probes recognize wild-type sequences, with the exception of the probe DET1136/mut. Fluo - fluorescein.

#### **Table 5:**

**[0016] Distribution of lipid and lipoprotein values in healthy individuals with different ABC1 polymorphisms.** HDL-C and ApoA-I levels in homozygous, heterozygous, and wild type male and female individuals are presented. Data are expressed as mean  $\pm$  SD. n indicates the number of analyzed individuals.

#### **Table 6:**

**[0017] Cholesterol and phospholipid efflux from mononuclear phagocytes isolated from healthy individuals homozygous for the wild type nucleotide at all four polymorphic sites and from healthy individuals ho-**

**mozygous for - the polymorphic allele at position 596 or 2589.** Lipid efflux is expressed as percent of basal unstimulated efflux observed in serum-free macrophage medium without ApoA-I. Data are the mean  $\pm$  SD of the indicated number (n) of different subjects. Efflux analysis for each subject was performed at least in quadruplicate. P values were calculated by independent Student T-test for wt/wt versus G596A and A2589G, respectively.

**Table 7:**

**[0018] Characteristics of FOS and VA-HIT subjects.** Results are listed as means  $\pm$  SD. \*Significantly different from male FOS control subjects,  $P < 0.01$ .

**Table 8:**

**[0019] Genotype distribution of the ABC1 polymorphisms in FOS versus VA-HIT subjects.** Values in parentheses are expressed as a percentage of the total number of subjects within each group.

**Table 9:**

**[0020] Association of the G596A and A2589G ABC1 polymorphisms in FOS and VA-HIT subjects.** Data represent absolute numbers of subjects out of a total of 1,008 for FOS and 1,014 for VA-HIT, whereas values in parentheses (%) provide the relative percentage of subjects within each group (FOS or VA-HIT). Significant differences in proportions between FOS and VA-HIT are presented in bold face type.

**Table 10:**

**[0021] Association of the G596A and G3456C ABC1 polymorphisms in FOS and VA-HIT subjects.** Data represent absolute numbers of subjects out of a total of 1,006 for FOS and 1,014 for VA-HIT, whereas values in parentheses (%) provide the relative percentage of subjects within each group (FOS or VA-HIT). Significant differences in proportions between FOS and VA-HIT are presented in bold face type.

**Table 11:**

**[0022] Association of the A2589G and G3456C ABC1 polymorphisms in FOS and VA-HIT subjects.** Data represent absolute numbers of subjects out of a total of 1,008 for FOS and 1,014 for VA-HIT, whereas values in parentheses (%) provide the relative percentage of subjects within each group (FOS or VA-HIT). Significant differences in proportions between FOS and VA-HIT are presented in bold face type.

Table 1:

Summary of the characteristics of the study population consisting of 516 healthy blood donors.						
	n	Age	Cholesterol mmol/l	Triglycerides mmol/l	HDL-C mmol/l	LDL-C mmol/l
Men	274	27.3 $\pm$ 7.7	4.95 $\pm$ 1.13	1.23 $\pm$ 0.85	1.43 $\pm$ 0.35	2.97 $\pm$ 1.01
Women	242	30.5 $\pm$ 10.2	5.63 $\pm$ 1.10	1.19 $\pm$ 0.56	1.72 $\pm$ 0.45	3.24 $\pm$ 1.02

Table 2:

Frequency of ABC1 Polymorphisms in healthy control subjects				
	n	Homozygous	Heterozygous	Wild Type
<b>G596A</b>	511	8.0 %	43.5 %	48.5 %
<b>A2589G</b>	514	2.3 %	22.8 %	74.9 %
<b>G3456C</b>	516	0.0 %	5.0 %	95.0 %
<b>T1136C</b>	515	0.0 %	0.6 %	99.4 %

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Table 3:

Distribution of G596A and A2589G Polymorphisms in healthy control subjects			
	G596A		
	596A/A	596G/A	596G/G
A2589G			
2589G/G	4 (9.8%)	6 (2.7%)	2 (0.8%)
2589A/G	14 (34.1%)	61 (27.6%)	39 (15.7%)
2589A/A	23 (56.1%)	154 (69.7%)	207 (83.5%)

Table 4:

Amplification primers and hybridization probes for the LightCycler polymorphism analysis	
G596A	
DET596/705 ANC596 FLA596-f FLA596-r	5'-LC Red 705-GCCTACCAAGGGAGAACTG-ph 5'-CTTGGTGACCAAGAAGTTTCTGAGCTTTG-Fluo 5'-TTTTGCAAGGCTACCAGTTACA 5'-CAGGATTGGCTTCAGGATGT
T1136C	
DET1136/mut ANC1136/B FLA1136-f FLA1136-r	5'-LC Red 640-ACTCCAGGCGAACAAGACC-ph 5'-TTTCTGCGGTCCCTGGCTCCCCACC-Fluo 5'-AAGTCTGATGCAGACCAGAGC 5'-CGGACAAGGTCCATTTCTTG
A2589G	
DET2589rev ANC2589rev FLA2589-f FLA2589-r	5'-LC Red 640-CTTTCTGATATTCTCTTCTGG-ph 5'-TTAAAGAAAGAGCAGGAGGTCAACAGCACT-Fluo 5'-AGGCCCTGGTATTTTCCTTG 5'-CCCTGGAGTGGTTTCACAGT
G3456C	
DET3456rev ANC3456rev FLA3456-f FLA3456-r	5'-LC Red 640-CGTGTCACTCTCATGGTCG-ph 5'-AGAAACCCCAGAGTCCTTACCGATGGTC-Fluo 5'-GGACAGTGTTTCTCAGAGCAG 5'-AGCAGCAAACCTTGAGTCAG

Table 5:

Distribution of lipid and lipoprotein values in healthy individuals with different <i>ABC1</i> polymorphisms			
	Homozygous	Heterozygous	Wild Type
G596A	596A/A	596G/A	596G/G
<b>men</b>	n=19	n=127	n=126
HDL-C (mmol/l)	1.41 ± 0.26	1.45 ± 0.36	1.41 ± 0.34
ApoA-I(g/l)	1.51 ± 1.6	1.49 ± 2.5	1.48 ± 2.3
<b>women</b>	n=22	n=95	n=122
HDL-C (mmol/l)	1.82 ± 0.45	1.75 ± 0.46	1.73 ± 0.44
ApoA-I(g/l)	1.77 ± 3.9	1.79 ± 3.6	1.76 ± 3.3
A2589G	2589G/G	2589A/G	2589A/A
<b>men</b>	n=7	n=54	n=212

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Table 5: (continued)

Distribution of lipid and lipoprotein values in healthy individuals with different <i>ABC1</i> polymorphisms			
	Homozygous	Heterozygous	Wild Type
A2589G	2589G/G	2589A/G	2589A/A
HDL-C (mmol/l)	1.50 ± 0.28	1.39 ± 0.32	1.44 ± 0.36
ApoA-I(g/l)	1.62 ± 21	1.47 ± 24	1.49 ± 24
<b>women</b>	n=5	n=63	n=173
HDL-C (mmol/l)	1.94 ± 0.40	1.80 ± 0.37	1.72 ± 0.48
ApoA-I(g/l)	2.02 ± 28	1.79 ± 32	1.76 ± 36
G3456C	3456C/C	3456G/C	3456G/G
<b>men</b>	n=0	n=15	n=228
HDL-C (mmol/l)		1.37 ± 0.27	1.44 ± 0.35
ApoA-I(g/l)		1.50 ± 26	1.49 ± 24
<b>women</b>	n=0	n=11	n=262
HDL-C (mmol/l)		1.84 ± 0.46	1.74 ± 0.45
ApoA-I (g/l)		1.79 ± 34	1.77 ± 35

Table 6:

Cholesterol and phospholipid efflux from mononuclear phagocytes isolated from healthy individuals homozygous for the wild type nucleotide at all four polymorphic sites and from healthy individuals homozygous for the polymorphic allele at position 596 or 2589.			
A. Cholesterol efflux: ApoA-I [10 µg/ml]			
	wt/wt 596G/G; 2589A/A	596A/A	2589G/G
<i>all probands</i>	128.7 ± 17.5% (n=18)	97.7 ± 22.4% (n=8; p<0.001)	103.4 ± 18.7% (n=11; p<0.001)
<i>men</i>	126.7 ± 16.6% (n=14)	98.8 ± 43.8% (n=2; n.s.)	104.5 ± 23.0% (n=6; p<0.025)
<i>women</i>	135.6 ± 21.4% (n=4)	97.3 ± 18.0% (n=6; p<0.001)	102.0 ± 14.3% (n=5; p<0.025)
B. Cholesterol efflux: ApoA-I [10 µg/ml]+ 24 h pretreatment with forskolin [10 µM]			
	wt/wt	596A/A	2589G/G
	596G/G; 2589A/A		
<i>men and women</i>	121.8 ± 16.4% (n=12)	126.7 ± 37.4% (n=8; n.s.)	121.5 ± 38.8% (n=10; n.s.)
C. Phospholipid efflux: ApoA-I [10 µg/ml]			
	wt/wt 596G/G; 2589A/A	596A/A	2589G/G
<i>men and women</i>	125.9 ± 15.3% (n=18)	117.3 ± 27.1% (n=8; n.s.)	114.9 ± 32.6% (n=10; n.s.)
D. Phospholipid efflux: ApoA-I [10 µg/ml]+ 24 h pretreatment with forskolin [10 µM]			
	wt/wt 596G/G; 2589A/A	596A/A	2589G/G
<i>men and women</i>	135.4 ± 20.2% (n=12)	136.7 ± 12.8% (n=8; n.s.)	124.5 ± 25.8% (n=10; n.s.)

Table 7:

Characteristics of FOS and VA-HIT subjects		
	FOS	VA-HIT
<b>N</b>	1014	1014
<b>Age (years)</b>	53±9	64±7*
<b>BMI (kg/m<sup>2</sup>)</b>	27.7±3.9	29±5*
<b>TC (mg/dL)</b>	206±36	175±25*
<b>LDL-C (mg/dL)</b>	136±33	111±23*
<b>HDL-C (mg/dL)</b>	44±12	32±5*
<b>TG (mg/dL)</b>	138±101	161±68
<b>TC:HDL-C</b>	5.1±1.6	5.7±1.1*

\*Significantly different from male FOS control subjects, P&lt;0.01.

Table 8:

Genotype distribution of the <i>ABC1</i> polymorphisms in FOS versus VA-HIT subjects			
Mutation	FOS	VA-HIT	P
G596A			
Total n	1013	1014	0.000
GG (%)*	543 (53.6)	454 (44.8)	
GA (%)	402 (39.7)	484 (47.7)	
AA (%)	68 (6.7)	76 (7.5)	
A Allele frequency	0.266	0.314	
A2589G			
Total n	1014	1014	0.001
AA (%)	779 (76.8)	707 (69.7)	
AG (%)	222 (21.9)	285 (28.1)	
GG (%)	13 (1.3)	22 (2.2)	
G Allele frequency	0.122	0.162	
G3456C			
Total <i>n</i>	1013	1014	0.032
GG (%)	965 (95.3)	940 (92.7)	
GC (%)	43 (4.2)	70 (6.9)	
CC (%)	5 (0.5)	4 (0.4)	
C Allele frequency	0.026	0.038	

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Table 9:

Association of the G596A and A2589G <i>ABC1</i> polymorphisms in FOS and VA-HIT subjects						
			G596A			
	<b>GG</b>		<b>GA</b>		<b>AA</b>	
	<u>FOS</u>	<u>VA-HIT</u>	<u>FOS</u>	<u>VA-HIT</u>	<u>FOS</u>	<u>VA-HIT</u>
A2589G						
<b>AA</b>	459 (45.6)	360 (35.5)	77 (7.6)	89 (8.8)	5 (0.5)	5 (0.5)
<b>AG</b>	268 (26.6)	30 (30.2)	125 (12.4)	166 (16.4)	5 (0.5)	12 (1.2)
<b>GG</b>	47 (4.7)	41 (4.0)	18 (1.8)	30 (3.0)	3 (0.3)	5 (0.5)

Table 10:

Association of the G596A and G3456C <i>ABC1</i> polymorphisms in FOS and VA-HIT subjects						
<b>G596A</b>						
	<b>GG</b>		<b>GA</b>		<b>AA</b>	
	<u>FOS</u>	<u>VA-HIT</u>	<u>FOS</u>	<u>VA-HIT</u>	<u>FOS</u>	<u>VA-HIT</u>
<b>G3456C</b>						
<b>GG</b>	508 (50.5)	426 (42.0)	27 (2.7)	26 (2.6)	3 (0.3)	2 (0.2)
<b>GC</b>	384 (38.2)	450 (44.4)	14 (1.4)	34 (3.4)	2 (0.2)	0 (0)
<b>CC</b>	66 (6.6)	64 (6.3)	2 (0.2)	10 (1.0)	0 (0)	2 (0.2)

Table 11:

Association of the A2589G and G3456C <i>ABC1</i> polymorphisms in FOS and VA-HIT subjects						
<b>A2589G</b>						
	<b>AA</b>		<b>AG</b>		<b>GG</b>	
<b>G3456C</b>						
<b>GG</b>	746 (74.0)	666 (65.7)	204 (20.2)	257 (25.3)	11 (1.1)	17 (1.7)
<b>GC</b>	26 (2.6)	39 (3.8)	15 (1.5)	27 (2.7)	1 (0.1)	4 (0.4)
<b>CC</b>	3 (0.3)	2 (0.2)	2 (0.2)	1 (0.1)	0 (0)	1 (0.1)

**Description of specific embodiments****Identification of four polymorphisms in the gene encoding the human ATP-binding cassette transporter 1 (*ABC1*) and association of these polymorphisms with decreased ApoA-I mediated efflux of cholesterol**

**[0023]** This invention provides the four polymorphisms G596A, T1136C, A2589G, and G3456C in the *ABC1* gene (*ABC1* gene see Figures 1 and 2), which affect the primary structure of the gene product. The frequencies of these *ABC1* polymorphisms were determined in a young and healthy population (Example 1).

**[0024]** The two most common polymorphisms (G596A and A2589G) are both associated with a decreased in vitro ApoA-I mediated efflux of cholesterol from mononuclear phagocytes, which is a typical feature of Tangier disease (Example 2).

**[0025]** No association of any of the polymorphisms were found with HDL-cholesterol, ApoA-I or any other lipoprotein fraction probably because the effect of the *ABC1* polymorphisms on the protein function is compensated by other factors controlling plasma HDL levels (Example 2 and 4).

**[0026]** The precise mechanism by which ABC1 promotes cellular lipid efflux is not completely defined. However, recently we could show that ABC1 is expressed on the plasma membrane and the Golgi complex, mediates ApoA-I associated export of cholesterol and phospholipids from the cell, and is regulated by cholesterol flux. Also, our results indicate that ABC1 is involved in lipid export processes involving vesicular budding between the Golgi and the plasma membrane. In addition to a possible function as direct lipid transporter, ABC1 may also be involved in the initiation of signal transduction processes stimulating intracellular lipid transport pathways (40). The finding that pretreatment of monocytes with forskolin, an cAMP elevating agent known to stimulate ApoA-I dependent lipid efflux (28), was able to normalize cholesterol and phospholipid efflux in 596A/A and 2589G/G subjects, may indicate that these gene variants affect the ABC1 function, which is related both to signaling and lipid transport. Since such a defect could be overcome by regulatory signals, this hypothesis may also provide a possible explanation for the lack of an effect on serum HDL levels.

**[0027]** In summary, these data emphasizes the role of ABC1 in lipid efflux, the first step in reverse cholesterol transport, and can be taken as evidence that common polymorphisms in *ABC1* directly affect cellular lipid homeostasis, which is a key factor in the atherogenic process.

**Frequencies of the identified *ABC1* gene polymorphisms in men with low HDL cholesterol levels and coronary heart disease**

**[0028]** The frequencies of three of the common single nucleotide polymorphisms in *ABC1* (G596A, A2589G, and G3456C) were found to be significantly increased in the population comprised of men having HDL deficiency and established CHD (participants enrolled in the Veterans Affairs Cooperative HDL Cholesterol Intervention Trial, VA-HIT) relative to control subjects from the Framingham Offspring Study (FOS). Moreover, associations were observed between *ABC1* polymorphisms and status, indicating that wild type alleles were consistently more prevalent in FOS than in VA-HIT but that mutant alleles were consistently more prevalent in VA-HIT (Example 3).

**[0029]** In summary, these data demonstrate that three of the common variants in the gene encoding ABC1 occur with significantly increased frequency in a population of men having low HDL-C as their primary lipid abnormality and established CHD. These results indicate that common mutations in the *ABC1* play a role in the development of CHD in the general population. These data also provide evidence for a role of *ABC1* in the premature CHD associated with common HDL deficiency states. All three *ABC1* variants are either conserved between human and mouse or are very similar as in the case of A2589G (isoleucine and valine, respectively), suggesting that each has an important functional role.

**[0030]** Furthermore, the identification of ABC1 as a transporter for IL-1 $\beta$  identifies this gene as a candidate gene for treatment of inflammatory diseases including rheumatoid arthritis and septic shock (27). The cytokine IL-1 $\beta$  is a broadly acting proinflammatory mediator that has been implicated in the pathogenesis of these diseases.

**[0031]** Moreover, we could demonstrate, that glyburide as an inhibitor of IL-1 $\beta$  secretion inhibits not only Caspase 1 mediated processing of pro-IL-1 $\beta$  and release of mature IL-1 $\beta$  but simultaneously inhibits ceramide formation from sphingomyelin mediated by neutral sphingomyelinase and thereby releases human fibroblasts from G<sub>2</sub>-phase cell cycle arrest. These data provide a further mechanism indicative for a function of ABC1 in signalling and cellular lipid metabolism.

**[0032]** Autoimmune disorders that are associated with the antiphospholipid syndrome (e.g. lupus erythematoses) can be related to dysregulation of B-cell and T-cell function, aberrant antigen processing, or aberrations in the asymmetric distribution of membrane phospholipids. ABC-transporters are, besides their transport function, candidate genes for phospholipid translocases, floppases and scramblases that regulate phospholipid asymmetry (outer leaflet: PC+SPM; inner leaflet: PS+PE) of biological membranes [11]. There is considerable evidence for a dysregulation of

members of the ABC-transporter family in patient cells. We conclude that these ABC-cassettes are also candidate genes for a genetic basis of antiphospholipid syndromes such as in Lupus erythematoses.

[0033] In summary, our findings emphasize how insight into the pathophysiology of a major disease process may be gleaned from a very rare genetic disorder.

[0034] Lipid disorders represent diseases characterized by changes in the lipid- and lipoprotein concentrations in the organism. Cardiovascular diseases represent diseases of the heart and vessel system including coronary heart disease and atherosclerosis. Inflammatory diseases include diseases such as psoriasis and lupus erythematoses.

[0035] This invention is directed to the nucleotide sequences of the identified *ABC1* polymorphism variants, the corresponding protein sequences, antibodies, pharmaceutical compositions, diagnostic tests, vectors, and vector host systems according to the claims.

[0036] Our findings identify *ABC1* as a target for the diagnosis of lipid disorders, cardiovascular diseases, and inflammatory diseases.

[0037] Our findings further identify *ABC1* as a target for therapeutic strategies aimed at the prevention of lipid disorders, cardiovascular diseases, and inflammatory diseases.

[0038] The identified *ABC1* polymorphisms may create immunogenic protein sites that can be used for the generation of antibodies. These antibodies can be used for the detection of mutated *ABC1*-protein and can be used for the diagnosis of lipid disorders, cardiovascular diseases, and inflammatory diseases.

[0039] Therapeutic strategies also include the gene therapy of lipid disorders, cardiovascular diseases, and inflammatory diseases. For this gene therapy, a polynucleotide consisting of a transcription unit is used. The transcription unit consists of at least 9 consecutive nucleotides that are identical or substantially similar to the *ABC1* cDNA sequence. Alternatively, the transcription unit consists of at least 9 consecutive nucleotides leading to the production of antisense RNA of wild type *ABC1* or *ABC1* containing one or more of the identified polymorphisms. The transcription unit is combined with heterologous transcription regulating sequences, which regulate the transcription in human cells. The heterologous transcription regulating sequences include a promoter that is constitutively active in human cells. Alternatively, the heterologous transcription regulating sequences can contain a promoter that can be induced or repressed in human cells by the addition of a regulatory substance.

[0040] Another therapeutic approach would be the application of antisense molecules or ribozymes or RNA decoys directed towards the *ABC1* transcripts resulting in a modulation of the *ABC1* expression. The methodology has been reviewed in (41).

[0041] This invention also includes a vector containing the above described transcription unit and transcription regulating sequences. Preferred vector is a virus, e.g. retrovirus, adenovirus, adeno-associated virus, herpes simplex virus, lentivirus, vaccinia virus, sindbis virus, semliki forest virus.

[0042] Preferred vectors are also plasmids.

[0043] The invention also includes a pharmaceutical preparation that contains the recombinant vectors, e.g. in a colloidal dispersion system. Preferred colloidal dispersion systems are liposomes or polylysine ligands.

[0044] The invention also includes pharmaceutical compositions that contain the above described recombinant vector constructs in combination with nontoxic, inert, pharmaceutically-suited carrier substances. Application of these pharmaceutical compositions can be locally or systemically (e.g. intravenous, intraarterial, intramuscular, subcutaneous, intradermal, anal, vaginal, nasal, transdermal, intraperitoneal, oral, or as aerosol).

[0045] The invention further includes recombinant host cells, especially recombinant eucaryotic host cells, that contain the above described recombinant vector constructs.

## Examples

### Example 1

#### ATP-binding cassette transporter 1 (*ABC1*) gene polymorphisms

#### Complete cDNA sequence of human *ABC1*

[0046] We have cloned the complete cDNA sequence of human *ABC1* (10). The nucleotide sequence of the complete coding region of the human *ABC1* gene is shown in Fig. 1. The open reading frame of 6603 bp encodes a 2201 amino acid protein with a predicted molecular weight of 220 kDa. The protein sequence of the human *ABC1* gene is shown in Fig. 2.

#### Identification of four polymorphisms in human *ABC1*

[0047] Complete sequence analysis (by dye primer sequencing) of human *ABC1* in different (how many?) Tangier



kindred's revealed several polymorphisms, which were also found in healthy control subjects. These are G596A, T1136C, A2589G, and G3456C. All of them change the amino acid sequence of ABC1 and therefore may affect the function of this transporter.

## Frequency of polymorphisms in the general population

[0048] In order to determine the frequency of these four polymorphisms in the general population, we have tested 516 healthy blood donors. Table 1 summarizes the characteristics of the blood donors. The allele frequencies shown in table 2 demonstrate that G596A, A2589G and G3456C variants are quite common in a young, healthy population. The G596A polymorphism had the highest prevalence with an estimated frequency of 29.8% for the minor allele (596A), followed by A2589G with 13.7% (2589G), and G3456C with 2.5% (3456C). There was no significant deviation from the Hardy-Weinberg equilibrium. Only only 3 heterozygotes were found for T1136C in our group.

[0049] When we further analyzed the distribution of the polymorphisms in healthy blood donors, we detected a frequent coincidence of the G596A and A2589G polymorphisms (table 3). In homozygote individuals for the minor G596A allele (596A/A; n=41) the frequency of the alleles A and G of the A2589G polymorphism was 73.2% and 26.8% respectively, while in homozygotes for the major G596A allele (596G/G; n=248) the frequencies were 91.3% and 8.7% ( $p < 0.001$ ). This indicates that the two mutations are in linkage disequilibrium in our population. While there was apparently no linkage disequilibrium between G596A and G3456C, the numbers were too small to exclude this with certainty (data not shown).

## Methods

[0050] *Sample collection.* Study subjects were recruited from healthy blood donors at the Institute for Clinical Chemistry (department of transfusion medicine) at the University of Regensburg. Samples from 516 individuals who gave consent to genetic analysis at the time of recruitment were available for the study. At the day of sample collection lipid parameters were determined and DNA-extraction was performed. A local review committee approved the Sample collection for genetic analysis.

[0051] *DNA extraction.* All DNA samples were isolated from whole blood with a QIAamp DNA Blood Maxi Kit (Qiagen) according to the manufacturer's recommendations.

[0052] *Detection of polymorphisms.* Genotype analysis was performed on the LightCycler (Roche Diagnostics) as previously described, with minor modifications (29,30). The amplification primers and hybridization probes are listed in table 4. The latter are designated as detection or anchor probes. They are labeled with LightCycler-Red 640 or 705 and fluorescein, respectively. We performed 40 amplification cycles under the following conditions: denaturation at 95°C for 120 s in the first step and 0 s in all subsequent steps, annealing at 57°C for 10 s, extension at 72°C for 15 s. This was followed by a temperature gradient melting curve analysis as described elsewhere (29,30). All detection probes recognize wild-type sequences, with the exception of the probe DET1136/mut, which is complementary to the sequence of the minor allele, i.e. 1136C. In order to assure specificity and accuracy of the LightCycler analysis 100 randomly selected samples were subjected to automated sequencing (ABI Prism 310 Genetic Analyzer), which proved full concordance of the results.

[0053] *Statistical Analysis.* To compare allele frequencies we employed chi-square test.

## Example 2

### **ABC1 gene polymorphisms are associated with impaired ApoA-I mediated cholesterol efflux but not with decreased serum HDL or ApoA-1**

[0054] In Tangier patients, mutations in *ABC1* are associated with severely decreased HDL-cholesterol and a lack or marked reduction of ApoA-I induced cholesterol and phospholipid efflux. Therefore, we hypothesized that HDL-cholesterol and ApoA-I levels as well as cholesterol and phospholipid efflux might differ between homozygous wild-type genotypes and carriers of one of the polymorphisms. Since the T1136C polymorphism was only found in heterozygous form in three individuals we did not include T1136C in our statistical analysis.

### **Analysis of the association of the identified ABC1 gene polymorphisms with HDL-cholesterol, ApoA-I, or other lipoprotein fractions**

[0055] We first analyzed the distribution of lipid and lipoprotein values in individuals with different genotypes. As indicated in table 5 neither heterozygous nor homozygous carriers of the G596A, A2589G, or G3456C polymorphisms had significantly different HDL-cholesterol or ApoA-I levels compared to the homozygous wild type probands. We also

analyzed further parameters of plasmatic lipoprotein metabolism, even though this was not an a priori hypothesis. Total plasma cholesterol, triglycerides, and LDL-cholesterol were also not significantly different among the various genotypes.

## 5 Analysis of the association of the identified *ABC1* gene polymorphisms with cholesterol and phospholipid efflux

[0056] We then analyzed cholesterol and phospholipid efflux from mononuclear phagocytes isolated from individuals homozygous for the wild type nucleotide at all four polymorphic sites and from individuals homozygous for the polymorphic allele at position 596 or 2589. Monocytes from subjects homozygous for at least one polymorphic allele were found to have a highly significant reduction in ApoA-I mediated cholesterol efflux (table 6 A). Whereas in cholesterol loaded monocytes from wild type individuals ApoA-I incubation for 17 hrs increases cholesterol efflux to approximately 130% of baseline values, no stimulation of efflux was observed in subjects homozygous for G596A (596A/A) or A2589G (2589G/G). This finding was independent of gender. In order to investigate, whether the lack of cholesterol efflux could be overcome by regulatory mechanisms, cells were pre-incubated with forskolin, a cAMP-elevating agent, which is known to stimulate ApoA-I mediated lipid efflux (28). As shown in table 6 B forskolin treatment normalized cholesterol efflux in subjects with polymorphic alleles.

[0057] A tendency towards reduced ApoA-I mediated efflux of choline containing phospholipids was also correlated with the presence of both variant alleles but did not reach statistical significance (table 6 C). Similar to the ApoA-I mediated cholesterol efflux, forskolin pretreatment abolished the differences between wild type and 596A/A or 2589G/G, respectively (table 6 D).

## Methods

[0058] *Lipids and Lipoproteins.* Venous blood was taken from all blood donors after a 12-h fast. The samples were obtained in polypropylene tubes, centrifuged at 4000 x g for 10 min. Cholesterol, triglycerides, and HDL-cholesterol was measured by standard clinical chemistry protocols as previously described. LDL cholesterol was calculated by the Friedewald equation. ApoA-I was determined on a BNA nephelometer (Dade-Behring) with reagents from Dade-Behring.

[0059] *Cellular Lipid Efflux.* Human peripheral blood cells were isolated from lithium-heparin treated blood samples of adult, healthy volunteers. The peripheral blood mononuclear cell fraction was separated by density gradient centrifugation over Ficoll-Histopaque 1.007 (Sigma). Cells positive for CD2, CD7, CD16, CD19 and CD56 were removed by appropriate magnetic beads (Dyna) leaving > 80% pure monocytes. Isolated monocytes were then cultured at a density of  $1.5 \times 10^5$  cells/mL in polystyrene culture dishes (100x15mm Petri dish, Falcon) in a serum-free macrophage medium (Macrophage-SFM, Gibco Life Technologies) supplemented with 50ng/mL human recombinant M-CSF (R&D Systems). After an overnight incubation cells were cholesterol loaded for 24 hrs by addition of enzymatically modified LDL (E-LDL; 40  $\mu$ g/ml), which was prepared as previously described (31). The incubation medium was further supplemented with  $^{14}$ C-cholesterol (1.5  $\mu$ Ci/ml) and  $^3$ H-choline (10 $\mu$ Ci/ml) in order to label cellular cholesterol and choline containing phospholipids. In a subset of experiments cells were stimulated with 10  $\mu$ M forskolin during the labeling period. After 24 hrs monocytes were washed and incubated for 17 hrs with or without 10  $\mu$ g/ml ApoA-I (Sigma) in macrophage medium containing 0.1 % bovine serum albumin and 50ng/mL M-CSF. After incubation the media were removed and centrifuged at 800 x g to precipitate any detached cells. Cells were lysed in 0.2% SDS. Lipids were extracted by liquid extraction according to the method of Bligh and Dyer (13).  $^3$ H and  $^{14}$ C radioactivities of the total lipid extracts were measured by liquid scintillation counting in order to distinguish between  $^3$ H labeled phospholipids and  $^{14}$ C-cholesterol. Lipid efflux was calculated as percentage of disintegrations per minute (dpm) in medium divided by the sum of dpm recovered from medium and cells. The ApoA-I specific efflux is expressed as a percent of basal unstimulated efflux observed in serum-free macrophage medium without ApoA-I.

[0060] *Statistical Analysis.* For comparison of lipid parameters and efflux data between wild type and polymorphisms the two-sample t test was used.

## Example 3

### Frequencies of the identified *ABC1* gene polymorphisms in men with low HDL cholesterol levels and coronary heart disease

[0061] To address the question whether the identified polymorphisms in *ABC-1* can provide insight into common HDL deficiency states and CHD risk, we compared the frequencies of three common *ABC1* variants (G596A, A2589G, and G3456C) between two different populations.

[0062] One population consisted of men participating in the Framingham Offspring Study (FOS), a long-term prospective evaluation of cardiovascular disease risk factors (32), whereas the other population consisted of men enrolled in the Veterans Affairs Cooperative HDL Cholesterol Intervention Trial (VA-HIT). VA-HIT, a multicenter study initiated in 1991, was designed to address the hypothesis that increasing HDL-C levels with gemfibrozil therapy would reduce the incidence of death from CHD and nonfatal myocardial infarction in men with established CHD, who had reduced HDL-C concentrations ( $\leq 40$  mg/dL; 1 mmol/L) and normal LDL-C levels (33). The characteristics of these two groups are provided in table 7. Men participating in VA-HIT were older, and slightly heavier, than men in FOS. With respect to plasma lipids, men in VA-HIT had significantly lower concentrations of plasma total cholesterol (-15%), HDL-C (-27%), and LDL-C (-18%), with the greatest difference between the groups noted in HDL-C levels. These differences resulted in a TC:HDL-C ratio which was significantly increased in VA-HIT relative to FOS. In control subjects from the FOS with those of participants in the VA-HIT.

#### Screening of the FOS and VA-HIT populations for the presence of the identified *ABC1* polymorphisms

[0063] In order to test the hypothesis that *ABC1* polymorphisms may play a role in common HDL deficiency states and, ultimately, CHD risk, genomic DNA from 1,014 men per FOS and VA-HIT population, respectively, was screened for the presence of three common single nucleotide polymorphisms in the *ABC1* gene (G596A, A2589G, and G3456C), using sequence-specific primers with the LightCycler System. The frequency distribution of each *ABC1* polymorphism in the FOS and VA-HIT populations is provided in table 8. With regard to the G596A polymorphism, the frequency of the mutant A allele was significantly increased ( $P=0.000$ ) in VA-HIT subjects (0.314) relative to FOS (0.266), translating into fewer wild type, but more heterozygous, individuals in the former group. This was similarly the case for the A2589G mutation in which the mutant G allele frequency was 0.162 in VA-HIT versus 0.122 in FOS ( $P<0.001$ ). The G3456C polymorphism was rare in both the FOS and VA-HIT populations; however, a significant increase ( $P<0.032$ ) in the frequency of the mutant C allele was observed in VA-HIT subjects (0.038 vs. 0.026). Thus, the frequency of each of these single nucleotide polymorphisms was significantly increased in a population comprised of men having HDL deficiency and established CHD.

#### Analysis of the associations between each *ABC1* polymorphism and status

[0064] We further evaluated these data for the presence of associations between each *ABC1* polymorphism and status. Associations between the G596A and A2589G, G596A and G3456C, and A2589G and G3456C *ABC1* variants in FOS and VA-HIT subjects are presented in tables 9-11, respectively. In each case, a significantly greater proportion of FOS subjects possessed a pair of wild type alleles relative to VA-HIT subjects. In contrast, a significantly greater proportion of VA-HIT subjects possessed either one or two mutant alleles for each *ABC1* variant. These data indicate that wild type alleles were consistently more prevalent in FOS than in VA-HIT, with mutant alleles consistently more prevalent in VA-HIT relative to FOS, suggesting that the occurrence of these *ABC1* variants may be linked.

#### Methods

[0065] **Subjects.** Subjects were men participating in either the Framingham Offspring ( $n=1,014$ ) or VA-HIT ( $n=1,014$ ) studies. The design and methods for FOS have been described elsewhere in detail (32), as have the rationale, design, and results of the VA-HIT study (33). FOS subjects were participants in cycle 4 of FOS and were free of clinical evidence of CHD. For VA-HIT, men were recruited at 20 Veterans Affairs medical centers throughout the United States. Eligibility for the trial required a documented history of CHD, an age of  $<74$  years, an absence of coexisting conditions, an HDL-C level of  $\leq 40$  mg/dL (1.0 mmol/L), an LDL-C of  $\leq 140$  mg/dL (3.6 mmol/L), and a triglyceride level of  $<300$  mg/dL (3.4 mmol/L). Since DNA was available from 1,014 men participating in the VA-HIT study, an equal number of men participating in cycle 4 of FOS was randomly selected from a total of 1,139 samples available for analysis. Informed consent was obtained from all subjects. Nearly all of the subjects in both groups were Caucasians. Information on alcohol consumption, smoking, blood pressure, body mass index (BMI), and diabetes were available for all subjects enrolled in these studies. Plasma lipid and lipoprotein data used in our analyses were obtained from FOS subjects who were not taking a lipid-lowering medication, while those from the VA-HIT subjects were obtained at baseline, prior to randomization to a treatment (gemfibrozil, 600 mg bid) or placebo group.

[0066] **DNA Isolation.** Genomic DNA was extracted from whole blood samples using either QIAamp mini kits (Qiagen) or Generation Capture Column® kits (Gentra Systems).

[0067] **Mutation detection.** Genotype analysis was performed using LightCycler technology (Roche Diagnostics), as described elsewhere in detail (28,34,35). Briefly, this method enables recognition of alterations in a nucleotide sequence through the detection of changes in fluorescence emission, dependent upon energy transfer between fluorophores on two adjacent hybridization probes. Both probes recognize the sequence of a specific amplicon. The de-

tection probe, labeled on its 5' end with LightCycler-Red 640 or 705, overlaps the site of a polymorphic nucleotide, whereas the anchor probe, carrying fluorescein at the 3' end, binds to the amplicon 1 to 3 nucleotides upstream of the detection probe. After PCR in the presence of target DNA, samples are briefly denatured at 95°C, rapidly cooled to 35-40°C, and, in the final step, heated gradually to 70-80°C. Differentiation between a wild type and polymorphic allele is possible, because the detection probe will detach at a different melting temperature dependent upon the presence of a mismatch between the probe and amplicon sequences.

**[0068]** *Statistical analyses.* To compare the FOS and VA-HIT populations, we employed chi-square tests for categorical measures and two-sample t test for continuous measures, using the SYSTAT statistical package.

#### Example 4

##### Analysis of the associations between each *ABC1* polymorphism and plasma lipid profiles in FOS

**[0069]** Analyses were also performed to test for potential associations between each *ABC1* polymorphism and plasma lipid profiles in FOS. Because the VA-HIT subjects were selected primarily on the basis of plasma lipid values and CHD, we did not test for associations in this group, which was, by design, more homogeneous than that of FOS. In FOS, no statistically significant relationships were detected between any of the 3 *ABC1* polymorphisms and plasma lipids (data not shown). This remained the case after adjustment for familial relationships, age, BMI, smoking, alcohol intake, and apolipoprotein E genotype. Although this result is somewhat incongruous with evidence for a role of *ABC1* in the regulation of plasma HDL-C levels (9,11-15), we believe that *ABC1* polymorphisms may directly influence intracellular cholesterol trafficking and, ultimately, reverse cholesterol transport. Support for this hypothesis is provided by the results presented in *Example 2* demonstrating that the homozygosity for either the G596A or A2589G polymorphism is associated with decreased cellular cholesterol efflux in healthy, young subjects. Moreover, because HDL-C levels are modulated by other genetic and environmental factors (7, 36), direct effects of *ABC1* polymorphisms on plasma HDL-C concentrations may not be readily apparent, especially in the heterozygous state.

#### Methods

**[0070]** *Measurement of plasma lipids and lipoproteins.* Blood samples were collected from subjects, after a 12-14 h fast, into tubes containing 0.1% EDTA. Plasma was isolated and frozen for subsequent analysis of plasma lipid and lipoprotein concentrations. Plasma total cholesterol and triglyceride concentrations were determined using enzymatic assays (37). Plasma HDL-C concentrations were measured after dextran sulfate-magnesium precipitation of apoB-containing lipoproteins (38), and LDL-C levels were calculated with the equation of Friedewald, Levy, and Fredrickson (39).

**[0071]** *Statistical analyses.* To evaluate the relationships between *ABC1* genotype and plasma lipids, we used analysis of covariance techniques, employing several different models to adjust for potential confounders. Age, BMI, smoking status, alcohol consumption, and apolipoprotein E genotype were included in these models. Loglinear models were used to examine associations between the 3 polymorphisms and status (FOS or VA-HIT). For all 3 models, statistically significant interactions were detected between *ABC1* genotype and status, indicating that differences existed between the FOS and VA-HIT groups with regard to the presence of mutant alleles. Specifically, for each *ABC1* polymorphism, those in the FOS group were more likely to be categorized as wild type, whereas VA-HIT subjects were more likely to be classified as heterozygous.

## SEQUENCE LISTING

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           35             40             45
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      Met Arg Lys Val Leu Arg Thr Leu Gln Gln Ile Lys Lys Ser Ser Ser
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	Thr Gln Gln Gln Phe Val Ala Leu Leu Trp Lys Arg Leu Leu Ile Ala	
	1265	1270 1275 1280
15	Arg Arg Ser Arg Lys Gly Phe Phe Ala Gln Ile Val Leu Pro Ala Val	
	1285	1290 1295
	Phe Val Cys Ile Ala Leu Val Phe Ser Leu Ile Val Pro Pro Phe Gly	
	1300	1305 1310
20	Lys Tyr Pro Ser Leu Glu Leu Gln Pro Trp Met Tyr Asn Glu Gln Tyr	
	1315	1320 1325
	Thr Phe Val Ser Asn Asp Ala Pro Glu Asp Thr Gly Thr Leu Glu Leu	
	1330	1335 1340
25	Leu Asn Ala Leu Thr Lys Asp Pro Gly Phe Gly Thr Arg Cys Met Glu	
	1345	1350 1355 1360
	Gly Asn Pro Ile Pro Asp Thr Pro Cys Gln Ala Gly Glu Glu Glu Trp	
	1365	1370 1375
30	Thr Thr Ala Pro Val Pro Gln Thr Ile Met Asp Leu Phe Gln Asn Gly	
	1380	1385 1390
	Asn Trp Thr Met Gln Asn Pro Ser Pro Ala Cys Gln Cys Ser Ser Asp	
	1395	1400 1405
35	Lys Ile Lys Lys Met Leu Pro Val Cys Pro Pro Gly Ala Gly Gly Leu	
	1410	1415 1420
	Pro Pro Pro Gln Arg Lys Gln Asn Thr Ala Asp Ile Leu Gln Asp Leu	
	1425	1430 1435 1440
40	Thr Gly Arg Asn Ile Ser Asp Tyr Leu Val Lys Thr Tyr Val Gln Ile	
	1445	1450 1455
	Ile Ala Lys Ser Leu Lys Asn Lys Ile Trp Val Asn Glu Phe Arg Tyr	
	1460	1465 1470
	Gly Gly Phe Ser Leu Gly Val Ser Asn Thr Gln Ala Leu Pro Pro Ser	
	1475	1480 1485
	Gln Glu Val Asn Asp Ala Thr Lys Gln Met Lys Lys His Leu Lys Leu	
	1490	1495 1500
	Ala Lys Asp Ser Ser Ala Asp Arg Phe Leu Asn Ser Leu Gly Arg Phe	
	1505	1510 1515 1520
55	Met Thr Gly Leu Asp Thr Arg Asn Asn Val Lys Val Trp Phe Asn Asn	
	1525	1530 1535

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Lys Gly Trp His Ala Ile Ser Ser Phe Leu Asn Val Ile Asn Asn Ala  
 1540 1545 1550  
 5 Ile Leu Arg Ala Asn Leu Gln Lys Gly Glu Asn Pro Ser His Tyr Gly  
 1555 1560 1565  
 Ile Thr Ala Phe Asn His Pro Leu Asn Leu Thr Lys Gln Gln Leu Ser  
 1570 1575 1580  
 10 Glu Val Ala Pro Met Thr Thr Ser Val Asp Val Leu Val Ser Ile Cys  
 1585 1590 1595 1600  
 Val Ile Phe Ala Met Ser Phe Val Pro Ala Ser Phe Val Val Phe Leu  
 1605 1610 1615  
 15 Ile Gln Glu Arg Val Ser Lys Ala Lys His Leu Gln Phe Ile Ser Gly  
 1620 1625 1630  
 Val Lys Pro Val Ile Tyr Trp Leu Ser Asn Phe Val Trp Asp Met Cys  
 1635 1640 1645  
 20 Asn Tyr Val Val Pro Ala Thr Leu Val Ile Ile Ile Phe Ile Cys Phe  
 1650 1655 1660  
 Gln Gln Lys Ser Tyr Val Ser Ser Thr Asn Leu Pro Val Leu Ala Leu  
 1665 1670 1675 1680  
 25 Leu Leu Leu Leu Tyr Gly Trp Ser Ile Thr Pro Leu Met Tyr Pro Ala  
 1685 1690 1695  
 Ser Phe Val Phe Lys Ile Pro Ser Thr Ala Tyr Val Val Leu Thr Ser  
 1700 1705 1710  
 30 Val Asn Leu Phe Ile Gly Ile Asn Gly Ser Val Ala Thr Phe Val Leu  
 1715 1720 1725  
 Glu Leu Phe Thr Asp Asn Lys Leu Asn Asn Ile Asn Asp Ile Leu Lys  
 1730 1735 1740  
 35 Ser Val Phe Leu Ile Phe Pro His Phe Cys Leu Gly Arg Gly Leu Ile  
 1745 1750 1755 1760  
 Asp Met Val Lys Asn Gln Ala Met Ala Asp Ala Leu Glu Arg Phe Gly  
 1765 1770 1775  
 40 Glu Asn Arg Phe Val Ser Pro Leu Ser Trp Asp Leu Val Gly Arg Asn  
 1780 1785 1790  
 Leu Phe Ala Met Ala Val Glu Gly Val Val Phe Phe Leu Ile Thr Val  
 1795 1800 1805  
 45 Leu Ile Gln Tyr Arg Phe Phe Ile Arg Pro Arg Pro Val Asn Ala Lys  
 1810 1815 1820  
 Leu Ser Pro Leu Asn Asp Glu Asp Glu Asp Val Arg Arg Glu Arg Gln  
 1825 1830 1835 1840  
 Arg Ile Leu Asp Gly Gly Gly Gln Asn Asp Ile Leu Glu Ile Lys Glu  
 1845 1850 1855  
 55 Leu Thr Lys Ile Tyr Arg Arg Lys Arg Lys Pro Ala Val Asp Arg Ile  
 1860 1865 1870

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Cys Val Gly Ile Pro Pro Gly Glu Cys Phe Gly Leu Leu Gly Val Asn  
 1875 1880 1885  
 5 Gly Ala Gly Lys Ser Ser Thr Phe Lys Met Leu Thr Gly Asp Thr Thr  
 1890 1895 1900  
 Val Thr Arg Gly Asp Ala Phe Leu Asn Arg Asn Ser Ile Leu Ser Asn  
 1905 1910 1915 1920  
 10 Ile His Glu Val His Gln Asn Met Gly Tyr Cys Pro Gln Phe Asp Ala  
 1925 1930 1935  
 Ile Thr Glu Leu Leu Thr Gly Arg Glu His Val Glu Phe Phe Ala Leu  
 1940 1945 1950  
 15 Leu Arg Gly Val Pro Glu Lys Glu Val Gly Lys Val Gly Glu Trp Ala  
 1955 1960 1965  
 Ile Arg Lys Leu Gly Leu Val Lys Tyr Gly Glu Lys Tyr Ala Gly Asn  
 1970 1975 1980  
 20 Tyr Ser Gly Gly Asn Lys Arg Lys Leu Ser Thr Ala Met Ala Leu Ile  
 1985 1990 1995 2000  
 Gly Gly Pro Pro Val Val Phe Leu Asp Glu Pro Thr Thr Gly Met Asp  
 2005 2010 2015  
 25 Pro Lys Ala Arg Arg Phe Leu Trp Asn Cys Ala Leu Ser Val Val Lys  
 2020 2025 2030  
 Glu Gly Arg Ser Val Val Leu Thr Ser His Ser Met Glu Glu Cys Glu  
 2035 2040 2045  
 30 Ala Leu Cys Thr Arg Met Ala Ile Met Val Asn Gly Arg Phe Arg Cys  
 2050 2055 2060  
 Leu Gly Ser Val Gln His Leu Lys Asn Arg Phe Gly Asp Gly Tyr Thr  
 2065 2070 2075 2080  
 35 Ile Val Val Arg Ile Ala Gly Ser Asn Pro Asp Leu Lys Pro Val Gln  
 2085 2090 2095  
 Asp Phe Phe Gly Leu Ala Phe Pro Gly Ser Val Pro Lys Glu Lys His  
 2100 2105 2110  
 40 Arg Asn Met Leu Gln Tyr Gln Leu Pro Ser Ser Leu Ser Ser Leu Ala  
 2115 2120 2125  
 Arg Ile Phe Ser Ile Leu Ser Gln Ser Lys Lys Arg Leu His Ile Glu  
 2130 2135 2140  
 45 Asp Tyr Ser Val Ser Gln Thr Thr Leu Asp Gln Val Phe Val Asn Phe  
 2145 2150 2155 2160  
 Ala Lys Asp Gln Ser Asp Asp Asp His Leu Lys Asp Leu Ser Leu His  
 2165 2170 2175  
 50 Lys Asn Gln Thr Val Val Asp Val Ala Val Leu Thr Ser Phe Leu Gln  
 2180 2185 2190  
 Asp Glu Lys Val Lys Glu Ser Tyr Val  
 2195 2200  
 55

**Claims**

1. A polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide as set forth in SEQ ID NO:1 with the sequence variants G596A or T1136C or A2589G or G3456C or any combination of these variants;

(b) a polynucleotide fragment of the polynucleotides of (a) containing the variants G596A or T1136C or A2589G or G3456C or any combination of these variants.

2. The polynucleotide of claim 1 wherein the polynucleotide is DNA.

3. A vector containing one or more of the polynucleotides of claim 1 and 2.

4. A host cell containing the vector of claim 3.

5. A process for producing a polypeptide comprising: expressing from the host cell of claim 4 the polypeptide encoded by said DNA.

6. An assay using one of the materials of claims 1 to 4 for the detection of the ABC1 gene transcripts.

7. An assay using one of the materials of claims 1 to 4 for the detection of the ABC1 variants G596A or T1136C or A2589G or G3456C.

8. A polypeptide selected from the group consisting of:

a) a polypeptide having the deduced amino acid sequence of SEQ ID NO:1 and

b) fragments, analogs and derivatives thereof containing the sequence variants of claim 2b.

9. An antibody capable to bind to the polypeptide of claim 8.

10. An assay using one of the materials of claims 8 and 9 for the detection of

(a) the ABC1 protein or fragments, analogs and derivatives thereof,

(b) the deduced protein or fragments, analogs and derivatives thereof from the ABC1 sequence variants G596A or T1136C or A2589G or G3456C .

11. A diagnostic kit for the detection of the ABC1 gene variants G596A or T1136C or A2589G or G3456C.

12. Modulator of polypeptides encoded by a polynucleotide comprising a member selected from the group consisting of:

(a) a polynucleotide as set forth in SEQ ID NO:1 and

(b) a polynucleotide fragment of the polynucleotide of (a) containing the sequence variants G596A or T1136C or A2589G or G3456C.

13. A pharmaceutical comprising the modulator of claim 12.

14. The modulation of ABC1 transcripts or proteins or fragments thereof by gene therapy.

15. The modulation of ABC1 transcripts or proteins or fragments thereof by antisense or ribozyme technology or RNA decoys.

Figure 1: cDNA of human *ABC1* gene

```

1  AAACCCCGTA ATTGCGAGCG AGAGTGAGTG GGGCCGGGAC CCGCAGAGCC
51  GAGCCGACCC TTCTCTCCCG GGCTGCCGCA GGGCAGGGCG GGGAGCTCCG
101 CGCACCAACA GAGCCGGTTC TCAGGGCGCT TTGCTCCTTG TTTTTCCTCC
151 GGTTCGTGTTT TCTCCCTTTC TCCGGAAGGC TTGTCAAGGG GTAGGAGAAA
201 GAGACGCAAA CACAAAAGTG GAAAACAGTT AATGACCAGC CACGGCGTCC
251 CTGCTGTGAG CTCTGGCCGC TGCCTTCCAG GGCTCCCGAG CCACACGCTG
301 GGGGTGCTGG CTGAGGGAAC ATGGCTTGTT GGCCTCAGCT GAGGTGCTG
351 CTGTGGAAGA ACCTCACTTT CAGAAGAAGA caaacatgtc agctgttact
401 ggaagtggcc tggcctctat ttatcttctt gatcctgac tctgttcggc
451 tgagctaccc accctatgaa caacatgaat gccattttcc aaataaagcc
501 atgcccctcg caggaaactc tccttgggtt caggggatta tctgtaatgc
551 caacaacccc tgtttccggt acccgactcc tggggaggct cccggagtgt
601 ttggaaactt taacaaatcc attgtggctc gcctgttctc agatgtctcg
651 aggtctcttt tatacagcca gaaagacacc agcatgaagg acatgctgca
701 agttctgaga acattacagc agatcaagaa atccagctca aacttgaagc
751 ttcaagattt cctgggtggc aatgaaacct tctctgggtt cctgtatcac
801 aacctctctc tcccaaagtc tactgtggac aagatgctga gggctgatgt
851 cattctccac aaggtatatt tgcaaggcta ccagttacat ttgacaagtc
901 tgtgcaatgg atcaaaatca gaagagatga ttcaacttgg tgacgagaag
951 gtttctgagc tttgtggcct accaaggagg aaactggctg cagcagagcg
1001 agtacttcgt tccaacatgg acatcctgaa gccaatcctg agaacactaa
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1501 aggtcatggc tgaggtgaac aagaccttcc aggaactggc tgtgttccat
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1751 taatggttct gtgtacacct ggagagaagc tttcaacgag actaaccagg
1801 caatccggac catatctcgc ttcatggagt gtgtcaacct gaacaagcta
1851 gaacccatag caacagaagt ctggctcatc aacaagtcca tggagctgct
1901 ggatgagagg aagtctctgg ctggtattgt gttcactgga attactccag
1951 gcagcattga gctgccccat catgtcaagt acaagatccg aatggacatt
2001 gacaatgtgg agaggacaaa taaaatcaag gatgggtact gggaccttgg
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2151 accgagaaga aaactgggtg ctatatgcaa cagatgccct atccctgtta
2201 cgttgatgac atctttctgc gggatgatgag ccggtcaatg cccctcttca
2251 tgacgctggc ctggatttac tcagtggctg tgatcatcaa gggcatcgtg
2301 tatgagaagg aggcacggct gaaagagacc atgcggatca tgggcttggg
2351 caacagcatc ctctgggtta gctggttcat tagtagcctc attcctcttc
2401 ttgtgagcgc tggcctgcta gtggtcatcc tgaagttagg aaacctgctg
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2551 acctggcagc agcctgtggg ggcacatctc acttcacgct gtacctgccc
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2801 gatgctgttt gacaccttc tctatggggt gatgacctgg tacattgagg
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2901 accaagtctc actgggttgg cgaggaaagt gatgagaaga gccaccttgg
2951 ttccaaccag aagagaatat cagaaatctg catggaggag gaacccaccc
3001 acttgaagct gggcgtgtcc attcagaacc tggtaaaagt ctaccgagat
3051 gggatgaagg tggctgtcga tggcctggca ctgaattttt atgagggcca
3101 gatcacctcc ttcttgggac acaatggagc ggggaagacg accaccatgt

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3151 caatcctgac cgggttgttc ccccgacct cgggcaccgc ctacatcctg  
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 3251 ctgtccccag cataacgtgc tgtttgacat gctgactgtc gaagaacaca  
 3301 tctggttcta tgcccgtctg aaagggtctc ctgagaagca cgtgaaggcg  
 3351 gagatggagc agatggccct ggatgttggg ttgccatcaa gcaagctgaa  
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 3601 cggacgtcct gggggacagg attgccatca totcccatgg gaagctgtgc  
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6551 caaagcccgg cggttcttgt ggaattgtgc cctaagtgtt gtcaaggagg
6601 ggagatcagt agtgcttaca tctcatagta tggagaagtg tgaagctctt
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6751 gaatagcagg gtccaaccgg gacctgaagc ctgtccagga tttctttgga
6801 cttgcatttc ctggaagtgt tccaaaagag aaacaccgga acatgctaca
6851 ataccagctt ccatcttcat tatcttctct ggccaggata ttcagcatcc
6901 tctcccagag caaaaagcga ctccacatag aagactactc tgtttctcag
6951 acaacacttg accaagtatt tgtgaacttt gccaggacc aaagtgatga
7001 tgaccactta aaagacctct cattacacaa aaaccagaca gtagtggacg
7051 ttgcagttct cacatctttt ctacaggatg agaaagtga agaaagctat
7101 gtatgaagaa tcctgttcac acggggtggc tgaaagtaaa gagggactag
7151 actttccttt gcaccatgtg aagtgttgtg gagaaaagag ccagaagttg
7201 atgtgggaag aagtaaaactg gatactgtac tgatactatt caatgcaatg
7251 caattcaatg

```

Figure 2: human *ABC1* gene – protein translation

```

AAACCCCGTAATTGCGAGCGAGAGTGAGTGGGGCCGGGACCCGAGAGCC
1  -----+-----+-----+-----+-----+ 50
GAGCCGACCCCTTCTCTCCCGGGCTGCGGCAGGGCAGGGCGGGGAGCTCCG
51  -----+-----+-----+-----+-----+ 100
CGCACCAACAGAGCCGGTTCTCAGGGCGCTTTGCTCCTTGTTTTTTCCCC
101 -----+-----+-----+-----+-----+ 150
GGTTCTGTTTTCTCCCCCTTCTCCGGAAGGCTTGTCAGGGGTAGGAGAAA
151 -----+-----+-----+-----+-----+ 200
GAGACGCAAAACAAAAGTGGAAAACAGTTAATGACCAGCCACGGCGTCC
201 -----+-----+-----+-----+-----+ 250
CTGCTGTGAGCTCTGGCCGCTGCCTTCCAGGGCTCCCGAGCCACACGCTG
251 -----+-----+-----+-----+-----+ 300
GGGGTGCTGGCTGAGGGAACATGGCTTGTTGGCCTCAGCTGAGGTTGCTG
301 -----+-----+-----+-----+-----+ 350
          M A C W P Q L R L L -
CTGTGGAAGAACCTCAGTTTCAGAGAGAACaaacatgtcagctgttact
351 -----+-----+-----+-----+-----+ 400
L W K N L T F R R R Q T C Q L L L -
ggaagtggcctggcctctatattatcttctgatcctgatctctgttcggc
401 -----+-----+-----+-----+-----+ 450
E V A W P L F I F L I L I S V R L -
tgagctaccacccctatgaacaacatgaatgccattttccaaataaagcc
451 -----+-----+-----+-----+-----+ 500
S Y P P Y E Q H E C H F P N K A -
atgccctctgcaggaacacttcttgggttcaggggattatctgtaatgc
501 -----+-----+-----+-----+-----+ 550
M P S A G T L P W V Q G I I C N A -
caacaacccctgtttccgttaccgactcctggggaggctcccgagttg
551 -----+-----+-----+-----+-----+ 600
N N P C F R Y P T P G E A P G V V -

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        ttggaactttaacaaatccattgtggctcgctgttctcagatgctcgg
601  -----+-----+-----+-----+-----+ 650
        G N F N K S I V A R L F S D A R -

        aggcttcttttatacagccagaaagacaccagcatgaaggacatgcgcaa
651  -----+-----+-----+-----+-----+ 700
        R L L L Y S Q K D T S M K D M R K -

        agttctgagaacattacagcagatcaagaaatccagctcaaacttgaagc
701  -----+-----+-----+-----+-----+ 750
        V L R T L Q Q I K K S S S N L K L -

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751  -----+-----+-----+-----+-----+ 800
        Q D F L V D N E T F S G F L Y H -

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        N L S L P K S T V D K M L R A D V -

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851  -----+-----+-----+-----+-----+ 900
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901  -----+-----+-----+-----+-----+ 950
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1001 -----+-----+-----+-----+-----+ 1050
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1101 -----+-----+-----+-----+-----+ 1150
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1151 -----+-----+-----+-----+-----+ 1200
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7251 -----+ 7260



European Patent  
Office

# PARTIAL EUROPEAN SEARCH REPORT

Application Number

which under Rule 45 of the European Patent Convention shall be considered, for the purposes of subsequent proceedings, as the European search report

EP 00 10 5820

DOCUMENTS CONSIDERED TO BE RELEVANT			
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
D,X	BODZIOCH M ET AL: "THE GENE ENCODING ATP-BINDING CASSETTE TRANSPORTER 1 IS MUTATED IN TANGIER DISEASE" NATURE GENETICS, NEW YORK, NY, US, vol. 22, no. 4, August 1999 (1999-08), pages 347-351, XP000889766 ISSN: 1061-4036 * page 349, column 2, line 3 *	1-11, 14, 15	C12N15/12 C12N15/11 C12N9/00 C12N5/10 C07K14/705 C07K16/28 C12Q1/68 G01N33/53 A61K48/00
D,X	BROOKS-WILSON A ET AL: "MUTATIONS IN ABC1 IN TANGIER DISEASE AND FAMILIAL HIGH-DENSITY LIPOPROTEIN DEFICIENCY" NATURE GENETICS, NEW YORK, NY, US, vol. 22, no. 4, August 1999 (1999-08), pages 336-345, XP000889767 ISSN: 1061-4036 * figures 7,8 *	8-10	
			TECHNICAL FIELDS SEARCHED (Int.Cl.7)
			C12N C07K C12Q G01N A61K
<b>INCOMPLETE SEARCH</b>			
<p>The Search Division considers that the present application, or one or more of its claims, does/do not comply with the EPC to such an extent that a meaningful search into the state of the art cannot be carried out, or can only be carried out partially, for these claims.</p> <p>Claims searched completely</p> <p>Claims searched incompletely</p> <p>Claims not searched</p> <p>Reason for the limitation of the search</p> <p>see sheet C</p>			
Place of search		Date of completion of the search	Examiner
THE HAGUE		5 December 2000	Lonnoy, O
CATEGORY OF CITED DOCUMENTS			
<p>X : particularly relevant if taken alone</p> <p>Y : particularly relevant if combined with another document of the same category</p> <p>A : technological background</p> <p>O : non-written disclosure</p> <p>P : intermediate document</p> <p>T : theory or principle underlying the invention</p> <p>E : earlier patent document, but published on, or after the filing date</p> <p>D : document cited in the application</p> <p>L : document cited for other reasons</p> <p>&amp; : member of the same patent family, corresponding document</p>			

EPC FORM 1503 (3.82) (P04607)



European Patent  
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INCOMPLETE SEARCH  
SHEET C

Application Number  
EP 00 10 5820

Claim(s) not searched:  
12,13

Reason for the limitation of the search:

Present claims 12 and 13 relate to a compound defined by reference to a desirable characteristic or property, namely that it is a modulator of polypeptides of the invention. The claims cover all compounds having this characteristic or property, whereas the application provides support within the meaning of Article 84 EPC and/or disclosure within the meaning of Article 83 EPC for no such compound. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Independent of the above reasoning, the claims also lack clarity (Article 84 EPC). An attempt is made to define the compound by reference to a result to be achieved. Again, this lack of clarity in the present case is such as to render a meaningful search over the whole of the claimed scope impossible. Consequently, no search can be carried out for such claims, the wording of which is a mere recitation of the results to be achieved.



European Patent  
Office

## PARTIAL EUROPEAN SEARCH REPORT

Application Number  
EP 00 10 5820

DOCUMENTS CONSIDERED TO BE RELEVANT			CLASSIFICATION OF THE APPLICATION (Int.Cl.7)
Category	Citation of document with indication, where appropriate, of relevant passages	Relevant to claim	
D,X	LAWN R M ET AL: "THE TANGIER DISEASE GENE PRODUCT ABC1 CONTROLS THE CELLULAR APOLIPOPROTEIN-MEDIATED LIPID REMOVAL PATHWAY" JOURNAL OF CLINICAL INVESTIGATION, NEW YORK, NY, US, vol. 104, no. 8, October 1999 (1999-10), pages R25-R31, XP000884782 ISSN: 0021-9738 * figures 2-5 *	8-10	
E	WO 00 18912 A (BAYER AG ; KLUCKEN JOCHEN (DE); SCHMITZ GERD (DE)) 6 April 2000 (2000-04-06) SeqIdNo.1: 100.0% identity in 6880 bp overlap with SeqIdNo.1 / SeqIdNo.2: 100.0% identity in 2201 aa overlap with SeqIdNo.2	8-10	TECHNICAL FIELDS SEARCHED (Int.Cl.7)
A	YOUNG S G ET AL: "THE ABCS OF CHOLESTEROL EFFLUX" NATURE GENETICS, US, NEW YORK, NY, vol. 22, no. 4, August 1999 (1999-08), pages 316-318, XP000889764 ISSN: 1061-4036		



European Patent  
Office

Application Number  
EP 00 10 5820

### CLAIMS INCURRING FEES

The present European patent application comprised at the time of filing more than ten claims.

- ☐ Only part of the claims have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims and for those claims for which claims fees have been paid, namely claim(s):
- ☐ No claims fees have been paid within the prescribed time limit. The present European search report has been drawn up for the first ten claims.

### LACK OF UNITY OF INVENTION

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

see sheet B

- ☐ All further search fees have been paid within the fixed time limit. The present European search report has been drawn up for all claims.
- ☒ As all searchable claims could be searched without effort justifying an additional fee, the Search Division did not invite payment of any additional fee.
- ☐ Only part of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the inventions in respect of which search fees have been paid, namely claims:
- ☐ None of the further search fees have been paid within the fixed time limit. The present European search report has been drawn up for those parts of the European patent application which relate to the invention first mentioned in the claims, namely claims:



European Patent  
Office

**LACK OF UNITY OF INVENTION  
SHEET B**

Application Number  
EP 00 10 5820

The Search Division considers that the present European patent application does not comply with the requirements of unity of invention and relates to several inventions or groups of inventions, namely:

1. Claims: 1-15 (all partially)

G596A allele of ABC1, recombinant methods, products,  
therapeutic, and diagnostic applications relating thereto.

2. Claims: 1-15 (all partially)

T1136C allele of ABC1, recombinant methods, products,  
therapeutic, and diagnostic applications relating thereto.

3. Claims: 1-15 (all partially)

A2589G allele of ABC1, recombinant methods, products,  
therapeutic, and diagnostic applications relating thereto.

4. Claims: 1-15 (all partially)

G3456C allele of ABC1, recombinant methods, products,  
therapeutic, and diagnostic applications relating thereto.

**ANNEX TO THE EUROPEAN SEARCH REPORT  
ON EUROPEAN PATENT APPLICATION NO.**

EP 00 10 5820

This annex lists the patent family members relating to the patent documents cited in the above-mentioned European search report.  
The members are as contained in the European Patent Office EDP file on  
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

05-12-2000

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 0018912 A	06-04-2000	AU 5980499 A	17-04-2000

EPO FORM P0459

For more details about this annex : see Official Journal of the European Patent Office, No. 12/82